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A PRELIMINARY STUDY OF MECHANORECEPTORS WITHIN THE ANTERIOR BYSSUS RETRACTOR MUSCLE OF *MYTILUS EDULIS* L.

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Few studies have been performed on the anatomy and neurophysiological behavior of sensory receptors in the muscles of molluscs. Turner and Nevius (1951) were the first to report on mechanoreceptor activity in the foot of *Ariolimax*, a gastropod. Mechanoreceptor activity has been recorded in the cephalopod (Gray, 1960) and gastropod mantle (Laverack and Bailey, 1963) and the gastropod buccal mass (Laverack, 1970; Kater and Rowell, 1973). Mellon (1969) has studied the possible role of stretch receptors in the swimming behavior of the scallop.

The present work has been carried out on the anterior byssus retractor muscle (ABRM) of *Mytilus edulis* L. This smooth muscle responds to direct current stimulation with a tonic contraction, which continues long after stimulation has ceased, while it gives a phasic contraction in response to stimulation with alternating current or repetitive pulses of direct current (Winton, 1937; Jewell, 1959).

Even though much work has been done, comprehending the underlying physiological mechanisms controlling phasic and tonic contraction in molluscan smooth muscle still remains a challenge. An approach that may elucidate these mechanisms is to record proprioreceptor responses from the ABRM in response to mechanical stimuli. Such an approach can provide an index of the type of information the animal receives about the state of these muscles; further analysis might reveal the contribution of this sensory information in the regulation of the contractile states mentioned previously. In this report certain electrophysiological characteristics of mechanoreceptors found in the ABRM are described.

Materials and Methods

Specimens of *Mytilus edulis* L. were collected at Ram Island, Connecticut, and stored in tanks of flowing sea water at 4° C. The collected mussels were 2–6 cm long. Experiments took place within six hours after each collection.

In order to record electrophysiological activity from mechanoreceptors within the ABRM of an intact animal, a special preparation was developed. The animals were mounted with the left valve up on a piece of rectangular wood (oak) cemented to the middle of the bottom of an open plastic box. Mounting was accomplished by securing the lower valve rigidly to the wood by screws passing through the holes drilled in it. The preparation was immersed in natural sea water at 6–10° C.

After mounting, a large hole was cut in the left (top) valve; this was followed by partial removal of the mantle, exposing part of the left ABRM, pedal ganglion,

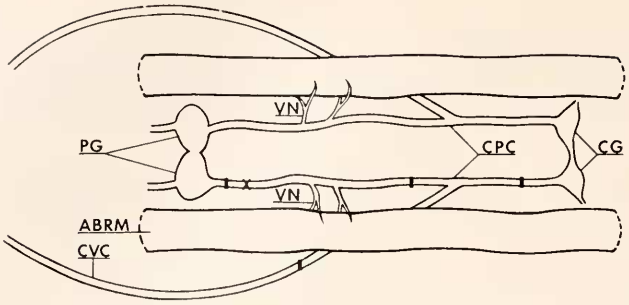


FIGURE 1. Isolation of the ABRM and its associated nerve, the cerebropedal connective nerve (CPC), from the nervous system. Bars represent the points at which the nerve was severed; and an X shows the point of hooking by an extracellular electrode.

cerebropedal connective (CPC) nerve, part of the foot, part of the cerebrovisceral connective nerve, and the visceral nerves.

At this point it was important to assess that the animals were in good condition. The criteria were that the mantle should not tear away from the shell upon light teasing of the tissue, and the foot, on being mechanically stimulated, should be sharply retracted. If these conditions were not met the animal was eliminated from the study. Any mussels with visible bacterial, fungal or nematode infestations were also not used.

By severing selected nerves at indentifiable points in the nervous system, the left ABRM and its innervating visceral nerves arising from the CPC were isolated from the rest of the nervous system (see Fig. 1). According to Twarog's (1960a) study, the innervation of the ABRMs comes entirely from the cerebral-pedal connectives, between the pedal ganglia and the branching of the cerebrovisceral connectives through the visceral nerves.

To measure muscle tension, a nonelastic cord was attached to a crop of byssus threads by surgical silk at one-half the length of the byssus threads. The other end of the nonelastic cord was attached to a force/displacement transducer.

Recording from the CPC nerve was accomplished by hooking the nerve with a platinum hook electrode insulated down to the hooked tip with GC Electronics Liquid-Tape. Drying of the CPC nerve was prevented by enveloping the hooked CPC nerve recording site with vasoline diluted to a workable consistency with parafin oil. The reference electrode, constructed the same way as the recording electrode except the bare tip was left without a hook, was placed near the byssal gland.

Mechanical stimulation was produced from a modified loud-speaker, driven by a signal generator with various amplitudes and frequencies. A plastic post from the middle of the loud-speaker impinged at right angles upon the nonelastic cord at one-half its length. Powdered molybdenum, a lubricant, was added to the point of contact between the probe of the modified loud-speaker and the nonelastic cord. The above method allowed direct measurement of tension on the ABRM. The signal generator which drove the loud-speaker also produced a synchronized trigger pulse which was recorded along with a muscle force on an FM tape recorder. Figure 2 illustrates the stimulating system.

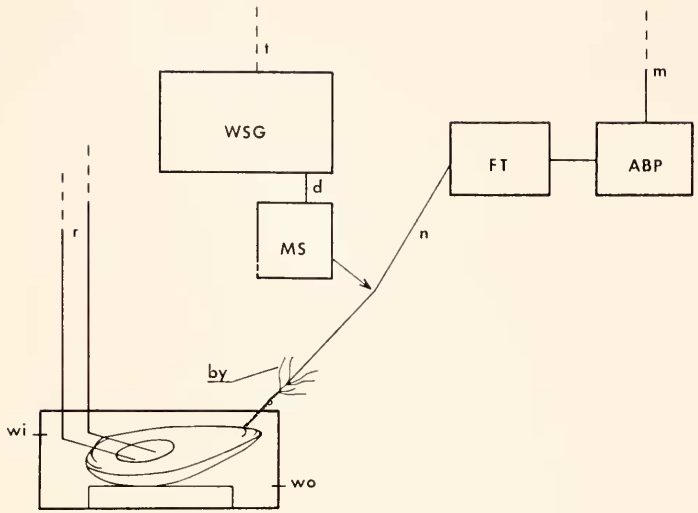


FIGURE 2. Schematic drawing of stimulating system. Abbreviations are: ABP, author built preamplifier; by, byssus threads; d, modified speaker's drive; FT, force transducer; MS, modified speaker; n, nonelastic cord; r, recording electrodes; wi, water in; wo, water out; and WSG, wavetek signal generator.

All electrophysiological recordings were amplified and then stored on a tape recorder. Conditioned data were then fed into a computer of average transients (signal averager) which produced synchronized stimulus histograms, or viewed directly on a strip-chart recorder or a storage oscilloscope.

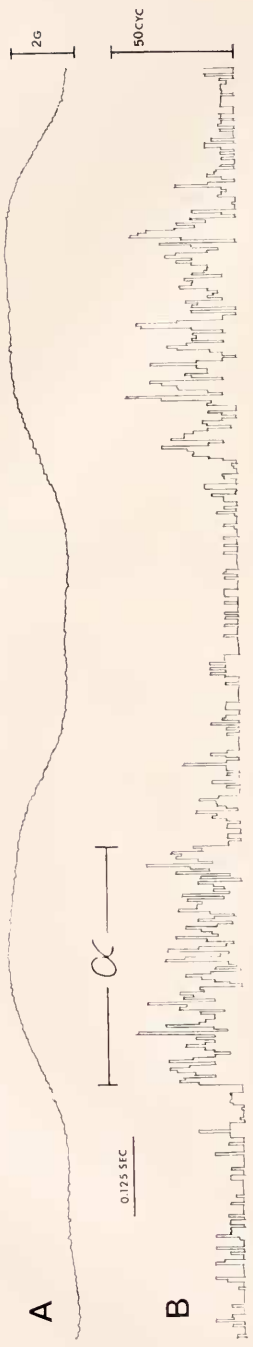
Measurement of a mussel's overall length was used for classification purposes instead of the ABRM's length because the preparation did not permit direct measurement of the ABRM's length.

RESULTS

Mechanical stimulation of an ABRM, neurally isolated except for its innervating visceral nerves and a portion of the CPC nerve, caused a multiunit discharge of impulses in the isolated CPC nerve, recorded by the platinum electrodes.

In order to determine the best stimulating frequency for further experimentation, the tension on the ABRM was varied sinusoidally around a mean value which was greater than the peak-to-peak (ppk) value of the sinusoid. Mussels of the same size were studied. Simultaneous, averaged records of tension and electrical activity of the mechanoreceptors over fifty cycles of each frequency were compiled by a signal averager. It was found that a 1.0-Hz mechanical stimulus produced the largest response of the mechanoreceptors. A 2.0-Hz mechanical stimulus is too fast for the sensory system to respond to, while a 0.1-Hz mechanical stimulus is too slow, not yielding a response over the background noise of the CPC nerve's spontaneous discharges. The activity of the mechanoreceptors due to a 0.5-Hz mechanical stimulus was not as large as the activity due to a 1.0-Hz stimulus.

Figure 3 shows a response of the mechanoreceptors to a 1.0-Hz sine wave


A
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2G
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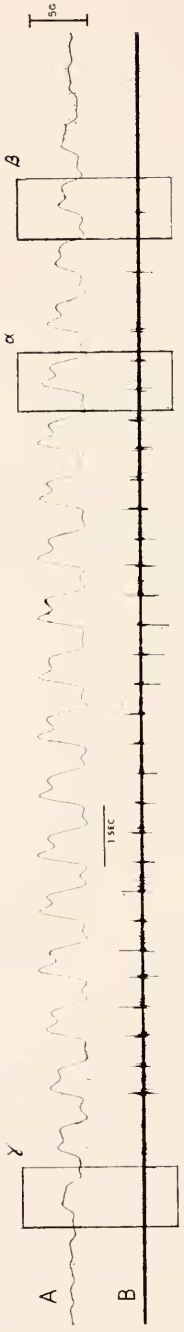
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mechanical stimulus which provides a ppk tension on the ABRM of 2 grams and a resting tension without external disturbances of 1.75 grams. It is seen from the record of the synchronized stimulus histogram that the mechanoreceptors produce firings between 1.75–2.75 grams (α of Fig. 3). These mechanoreceptors appear to be sensing stretch activity.

A response of the mechanoreceptors to a 1.0-Hz square wave stimulus is illustrated in Figure 4. The overshoot that appears on the square wave is probably due to the viscoelastic properties of the ABRM interacting with the stimulating loud speaker. The tension of the ABRM starts at rest at 6 grams, increases slowly through a few cycles of stimuli to a ppk tension of 4 grams and then finally decreases to the resting tension again. It is shown that some mechanoreceptors fire between 4.25–4.50 grams (*e.g.*, α of Fig. 4) with rising tension and others fire between 6.75–7.0 grams (*e.g.*, α of Fig. 4) falling tension. As can be seen in Figure 4, a threshold for firing can be shown as the amplitudes and rate of tension increases or decreases; as firing is absent (β and γ of Fig. 4) at lower levels of tension.

In all, three preparations each of four different mussel sizes were investigated. In the preparations, the resting tensions, the ppk values of the sine and square wave stimuli were varied but the stimulating frequency remained at 1.0-Hz. It was found that the mechanoreceptors' firings were repeatable, as shown in Figure 4. Threshold levels of tension for mechanoreceptors' firings were found to vary in position and number in relation to the animal's size.

The mechanoreceptors' responses to loading are distinct from those responses to unloading. As shown in Figure 5, the receptors responding to the rising phase (+) have spikes of large amplitude, while those responding to the falling phase (−) have lower amplitude spikes. These responses are repeatable. This evidence suggests that there are two functionally independent sets of receptors in the ABRMs which respond either to stretch or the release of tension.

Discussion

Mechanically sensitive receptor units have been found in the ABRM of *Mytilus edulis* L. The receptors responsible for the electrophysiological sensory activity within the ABRM may be the same putative receptors described morphologically by Gilloteaux (1971, 1972) as "neuro-muscular associations." Gilloteaux (1972) states that each neuro-muscular association consists of a nerve branch forming the nerve ending, generally in the form of a large spiral rolled around the modified smooth muscle fiber. Similar neuro-muscular structures were observed morphologically by Coujard (1950) in the Amerbach intestinal plexus of the rat and of the African lung-fish, *Protopterus*. These neuromuscular associations were interpreted (Gilloteaux, 1971, 1972) as interoceptors. Analogous neuromuscular associations have been observed using methylene blue staining techniques under the

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Figure 3. [On left.] Mechanoreceptors' responses to sine wave stimuli: (A) mechanical stimulus; (B) synchronized histogram. The mussel size is 4.3 cm.

Figure 4. [On right.] Mechanoreceptors' activity: (A) mechanical stimulus; (B) sensory activity of the mechanoreceptors due to square wave stimuli. The mussel length is 4.3 cm.

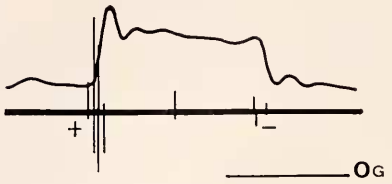

FIGURE 5. Independence of mechanoreceptors' firing through rising and falling tension. Rising tension receptors indicated by a positive sign; fire with larger amplitude than falling tension receptors indicated by a negative sign. The total sweep is one sec, and the stimulus is one cycle per second with a resting tension of 6 grams and a ppk value of 4 grams.

light microscope by LaCourse (1977) in the ABRM of *Mytilus*. In addition to Gilloteaux's description of the association, LaCourse has observed a bipolar cell, just before the nerve of the neuromuscular association terminates in the muscle fiber. LaCourse (1977) speculated that the bipolar cell is probably the soma of a sensory neuron, and the total bipolar cell with the neuromuscular structure is a mechanoreceptor. Similar bipolar cells have been observed embedded in connective tissue around the shafts of thick apodemes of the tailspine muscle of *Limulus polyphemus* (Eagles and Hartman, 1975). Eagles and Hartman speculated that these bipolar cells are tension receptors.

It is suggested by the present authors that the ABRM contains mechanoreceptors. These sensory receptors shown in the present electrophysiological study may arise from the morphologically observed neuromuscular associations described above.

From the results of the present investigation and evidence cited in the previous discussion, a speculative model for phasic contraction of the ABRM of *Mytilus* is offered. Figure 6 is a schematic representation of the model. The contractor innervation is assumed to arise in or beyond the pedal ganglion (from morphological observation of nerve tracts connecting the pedal nerve to the cerebropedal connective nerve, LaCourse, 1977), pass anteriorly along the cerebropedal connective nerves, through the visceral nerves to the middle third of the ABRM. There it branches into a fine nerve plexus covering the muscle fibers. When the motor nerves are stimulated, the plexus releases acetylcholine which acts directly on the muscle membrane to cause depolarization and contraction (Twarog, 1954, 1960b, 1967b). In turn, the mechanoreceptors located on the muscle fibers elicit potentials that are proportional to the stretch (rising or falling) on the ABRM. The elicited receptor firing is fed back to the pedal ganglion for further motor control.

The relaxor nerve fibers follow the same pathway to the muscle as the contractor innervation. When muscle relaxation is ordered, relaxing nerves are stimulated. Five-hydroxytryptamine is released from these nerves, causing relaxation of the muscle fibers (Twarog, 1954, 1960b, 1967a; Northrop, 1964). The effect of 5-hydroxytryptamine on the mechanoreceptors is not known at this time.

In essence, it is hypothesized that the mechanoreceptors are part of a sensory feedback system which supplies information to the pedal ganglion for motor control of the ABRMs. This investigation has raised interesting questions about the details of the ABRMs motor control system. It is evident that much work has yet

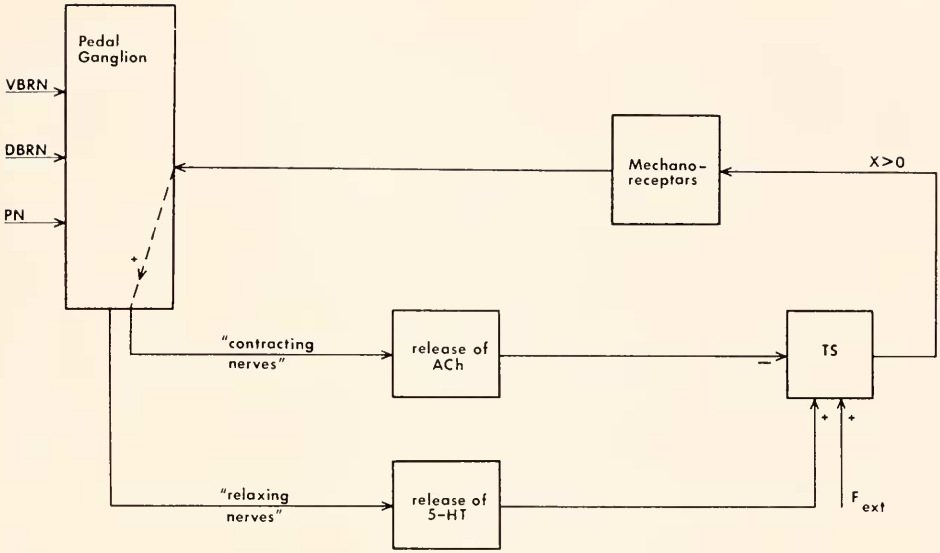

FIGURE 6. Proposed model for phasic control of ABRM. Abbreviations are: TS, nonlinear transfer function, relating muscle length to tension; F, external load; $X > 0$ is muscle lengthening; pluses on TS shows parametric control, VBRN, ventral byssus retractor nerve; DBRN, dorsal byssus retractor nerve; and, PN, pedal nerve.

to be done to clarify the closed-loop neurophysiological mechanism involved, and its relation to the so-called catch state of the ABRM.

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SUMMARY

1. Electrophysiological studies of mechanoreceptors within the ABRM of the mussel, *Mytilus edulis* have revealed two types of tension receptors. Multiunit recordings were made from an isolated nerve-muscle preparation during stretch with platinum hook electrodes. The responses observed were: type one, showing receptor firing during the rising phase of applied tension and type two, showing receptor firing during the falling phase. Based on electrical waveforms of multiunit recordings, both types of receptors seem to fire independently of each other, and can be reliably excited by the stimulus.

2. These mechanoreceptors are suggested to be part of a system which supplies information to the pedal ganglion for motor control of the ABRM.

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